Treatment of Severe Forms of LPS-Responsive Beige-like Anchor Protein (LRBA) Deficiency by Allogeneic Hematopoietic Stem Cell Transplantation

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Capsule summary

LRBA deficiency, a life-threatening immunodeficiency/autoimmunity disorder, was treated by HSCT in 12 patients, four of whom died from transplant-related causes. Six patients were cured without further need of immunosuppression, and two are in partial remission.

To the Editor

LPS-responsive beige-like anchor protein (LRBA)-deficiency is a severe primary immunodeficiency with a variable clinical phenotype, including features overlapping with common variable immunodeficiency, autoimmune lymphoproliferative syndrome, immune dysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome, and an association with lymphoma[1-3]. Despite accumulating experience, the procedure of allogeneic hematopoietic stem cell transplantation (HSCT) to treat primary multi-organ autoimmunity disorders and combined immunodeficiencies with immune dysregulation such as LRBA deficiency is not routine. First, the indication and optimal time point are undetermined. Second, the outcome of HSCT in syndromes with predominant autoimmunity is unclear, given that target antigens of autoimmune reactions remain unchanged, and autoimmunity as well as inflammation may persist due to disease-causing factors extrinsic to the hematopoietic and immune system. For instance, LRBA is a ubiquitously-expressed protein functioning in autophagy and intracellular vesicle trafficking, facilitating cell surface translocation of CTLA4[4, 5]. Its relevance in tissues other than immune cells is unknown. Augmentation of regulatory T cell function in LRBA-deficient individuals manifesting with chronic enteropathy by regular CTLA4-Ig infusions potentially induces remission[4]. However, not all symptoms respond equally, and patients remain dependent on CTLA4 substitution. Furthermore, the additional use of steroids, sirolimus, hydroxychloroquine, and other immunosuppressants may not prevent the long-term deterioration of patients with this potentially fatal disease. Finally, CTLA4-Ig is not available in many countries due to limited resources. Although HSCT has proven helpful in single patients[1, 6-9], early reports of HSCT in cases of LRBA deficiency indicated a higher transplant-related mortality (TRM) than in other inborn errors (2 of first 4 patients); it is unclear whether disease status at time of HSCT or other disease- or transplant-related factors were involved. To better understand the role of HSCT as a potentially curative treatment for LRBA deficiency, we performed an international EBMT-, and ESID-wide survey in 2016 to collect information about the HSCT experience. Data were obtained in accordance with the Declaration of Helsinki and an Institutional Board review (IRB00002556, 29-142ex16/17) by retrospective chart review by the local physicians. From a cohort of 72 patients with LRBA deficiency, 12 underwent HSCT between 2005-2016 (Table 1). Indications for transplant included refractory immune cytopenias, gastrointestinal

problems, parenchymal lung disease, failure to thrive, severe neurological or infectious complications, or plainly “severe course of the disease” (Table 1 and Figure 1A). Overall survival of transplanted patients was 67% (8/12 patients); all deaths were due to TRM (pre-existing infections, graft failure, multi-organ failure, thrombotic microangiopathy) and occurred within three months of HSCT (Table 1, Figure 1, and Supplementary Figure 1). Eleven donors were HLA-matched unrelated or family donors (4 and 7, respectively). One of the deceased patients was the only subject of the cohort with a mismatched (haploidentical family) donor. Surviving patients showed mostly favorable degrees of remission (complete: 4; good partial: 2 [some mild or moderate potentially LRBA-related symptoms, not requiring immunosuppressive therapy]; partial: 2 [amelioration of disease but need of immunosuppression for potentially LRBA-related symptoms]) with a median follow-up of 26 months (range 4-142; Table 1 and Figure 1). HSCT course and recurrence or persistence of symptoms were apparently not dependent on the donor's LRBA status or on type/intensity of conditioning. All survivors in complete or good partial remission showed full donor chimerism (>95% donor white blood cells, WBC). Two patients with partial remission (requiring immunosuppressive treatment) showed declining donor WBC chimerism <90%: patient 3 had 89% donor WBC and 88% donor T cells on day +240, further declining to 83 and 84%, respectively, on day +720, and was treated with sirolimus and romiplostim for moderate immune thrombocytopenia and autoimmune hemolytic anemia (AIHA) that developed on days +90 and +270, respectively. Patient 9, with only 7% WBC and 23% T cell chimerism on day +578, was successfully treated with abatacept after attempts with steroids and rituximab for AIHA (see details in Table 1). Both patients are clinically stable under the current treatment and did not develop other autoimmune symptoms they previously showed due to LRBA deficiency (Fig. 1A, Table 1). Thus, best results of HSCT for LRBA deficiency were associated with full donor chimerism. Donor source, transplant regimen, GvHD incidence, and disease response are listed in Table 1 and Figure 1. Strikingly, LRBA-related symptoms resolved or decreased significantly in number and intensity and performance scores increased in all HSCT survivors (Figure 1A-C). Consequently, the need for immunosuppressive treatment declined and most patients were off immunoglobulin replacement post-HSCT (Figure 1D-E). Unfortunately, there are no up-to-date follow-up and survival data of untransplanted patients beyond previous publications of large cohort

studies, that reported at least six deceased of 58 untransplanted but many newly diagnosed patients and family members[1, 2, 5].

Although the number of patients surveyed was too small to routinely recommend HSCT for LRBA-deficiency based on the evidence collected, these data support the feasibility and curative potential of HSCT and allow us to state three important points.

First, remissions without further need for immunosuppression are achievable and occurred in 6 of the 8 surviving patients. No relapse occurred in patients with full donor chimerism. Importantly, although the treatment-requiring autoimmune cytopenias in patients 3 and 9 observed after HSCT are possibly caused by LRBA deficiency due to waning donor chimerism, they could also be attributed to graft failure, post-HSCT immune dysregulation, or marrow dysfunction, like suspected in the growth factor responsive thrombocytopenia and neutropenia of patient 11, as such sequelae may similarly occur in HSCT recipients with other underlying diseases. With a median follow-up of 2 years, six of eight patients did not need further pharmacological immunosuppression or immunoglobulin replacement therapy, indicating curability. Only one patient in this cohort received abatacept as bridging treatment to HSCT. It is unclear whether response to abatacept would have changed the HSCT indication. However, despite the overall satisfying responses to abatacept (reported in 10 patients in the cohort of 72 patients), the long-term dependency on this treatment with its associated potential risks remains. The risk of LRBA-related lymphoma or immunosuppression-associated malignancy would be anticipated to be substantially reduced or abolished in HSCT survivors, as well as the future risk of infections, as compared to patients undergoing continuous treatment with CTLA4-Ig, sirolimus or other immunosuppression. Thus, based on the present results, we would proceed to HSCT if a suitable donor is present. The optimal time point remains to be determined, but it can be expected that long-term organ damage will impact negatively on HSCT results.

Second, among the small cohort of 12 LRBA-deficient patients who were transplanted from heterozygous LRBA mutation carriers (n=6), LRBA wild-type or unrelated donors (n=5), no correlation between residual or recurring LRBA-related symptoms and donor LRBA status was detected, arguing against a dosage effect of LRBA[2, 6].

Lastly, these data indicate that any alluded LRBA-dependent effects outside the (transplanted) hematopoietic/immune tissues play no or only an inferior role with regard to outcome and remission.

Together, accepting the limitation that the retrospective case series lacked a matched control group, our findings strongly support the use of early HSCT in patients with severe presentations of LRBA deficiency.

Supplemental Data are available in the Journal's Online Repository at www.xxxyyyzzz.org**.**

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References

1. Gámez-Díaz L, August D, Stepensky P, Revel-Vilk S, Seidel MG, Noriko M, et al. The extended phenotype of LRBA deficiency. Frontiers in Immunology 2015.
2. Alkhairy OK, Abolhassani H, Rezaei N, Fang M, Andersen KK, Chavoshzadeh Z, et al. Spectrum of Phenotypes Associated with Mutations in LRBA. J Clin Immunol 2016; 36:33-45.
3. Charbonnier LM, Janssen E, Chou J, Ohsumi TK, Keles S, Hsu JT, et al. Regulatory T-cell deficiency and immune dysregulation, polyendocrinopathy, enteropathy, X-linked-like disorder caused by loss-of-function mutations in LRBA. J Allergy Clin Immunol 2015; 135:217-27.
4. Lo B, Zhang K, Lu W, Zheng L, Zhang Q, Kanellopoulou C, et al. AUTOIMMUNE DISEASE. Patients with LRBA deficiency show CTLA4 loss and immune dysregulation responsive to abatacept therapy. Science 2015; 349:436-40.
5. Lopez-Herrera G, Tampella G, Pan-Hammarstrom Q, Herholz P, Trujillo-Vargas CM, Phadwal K, et al. Deleterious mutations in LRBA are associated with a syndrome of immune deficiency and autoimmunity. Am J Hum Genet 2012; 90:986-1001.
6. Seidel MG, Hirschmugl T, Gamez-Diaz L, Schwinger W, Serwas N, Deutschmann A, et al. Long-term remission after allogeneic hematopoietic stem cell transplantation in LPS-responsive beige-like anchor (LRBA) deficiency. J Allergy Clin Immunol 2015; 135:1384-90 e8.
7. Tesi B, Priftakis P, Lindgren F, Chiang SC, Kartalis N, Lofstedt A, et al. Successful Hematopoietic Stem Cell Transplantation in a Patient with LPS-Responsive Beige-Like Anchor (LRBA) Gene Mutation. J Clin Immunol 2016; 36:480-9.
8. Sari S, Dogu F, Hwa V, Haskologlu S, Dauber A, Rosenfeld R, et al. A Successful HSCT in a Girl with Novel LRBA Mutation with Refractory Celiac Disease. J Clin Immunol 2016; 36:8-11.
9. Bakhtiar S, Gamez-Diaz L, Jarisch A, Soerensen J, Grimbacher B, Belohradsky B, et al. Treatment of Infantile Inflammatory Bowel Disease and Autoimmunity by Allogeneic Stem Cell Transplantation in LPS-Responsive Beige-Like Anchor Deficiency. Front Immunol 2017; 8:52.

Legends

Table 1. Clinical characteristics and laboratory parameters of 12 LRBA-deficient patients who underwent allogeneic hematopoietic stem cell transplantation (HSCT). *Abbreviations*: (+3yrs) or (d+84) ,time point after HSCT; ADEM, acute disseminated encephalomyelitis; AdV, adenovirus; aGvHD, acute graft-versus-host disease; AIHA, autoimmune hemolytic anemia; AZT, azathioprine; cGvHD, chronic graft-versus-host disease; CMV, cytomegalovirus; CNS, central nervous system; CR, complete remission; CSA, cyclosporine A; CVID, common variable immunodeficiency; FK, tacrolimus/FK506; FU, follow-up; GCSF, granulocyte colony stimulating factor; GPR, good partial remission (mild residual symptoms- potentially LRBA-related- but without requirement of immunosuppressive treatment); het, heterozygous; IDDM, insulin-dependent diabetes mellitus –in this case as sequelae after pancreatitis; IS, immunosuppressive drugs; IVIG, regular intravenous immunoglobulin replacement therapy;LRBA, lipopolysaccharid-responsive beige-like anchor protein; MFD, matched family donor; MMF, mycophenolate mofetil; MMFD, mismatched family donor; MSD, matched sibling donor; MTX, metothrexate; MUD, matched unrelated donor; n.d. ,no data or not determined; PR, partial remission (moderate residual symptoms- potentially LRBA-related- with requirement of immunosuppressive treatment); SC boost, stem cell boost; SCIG, regular subcutaneous immunoglobulin replacement therapy; steroids, glucocorticoids; T, B, T and B lymphocytes; TMA, thrombotic microangiopathy; TRA, thrombopoietin receptor agonist treatment; TRM, transplant-related mortality; WBC, white blood cells; wt, wild-type.

Figure 1. Qualitative and semiquantitative assessment of disease activity of 12 patients with LRBA deficiency before and after hematopoietic stem cell transplantation. ***A,*** A heat map of LRBA-related symptoms is shown indicating symptom severity in patients before and after HSCT in pairs of columns (white: absent; light grey, mild-moderate; dark grey: severe and considered as HSCT indication; black columns indicate deceased patients). ***B,*** number of symptoms (as listed in *A*) before and after HSCT. ***C,*** Karnovsky/Lansky performance scores before and after HSCT; deceased patients were excluded from the analysis after HSCT. ***D,*** number of immunosuppressive drugs used simultaneously or sequentially for the treatment of LRBA-related symptoms before and after HSCT. ***E,*** immunoglobulin therapy shown as

194 *none* (bottom line); sporadic, indicating use as irregular immunomodulatory treatment
195 (center level); or regular, indicating monthly intravenous or weekly subcutaneous
196 replacement therapy (top).
197
198

Table 1. Clinical characteristics and laboratory parameters of 12 LRBA-deficient patients who underwent allogeneic hematopoietic stem cell transplantation (HSCT).

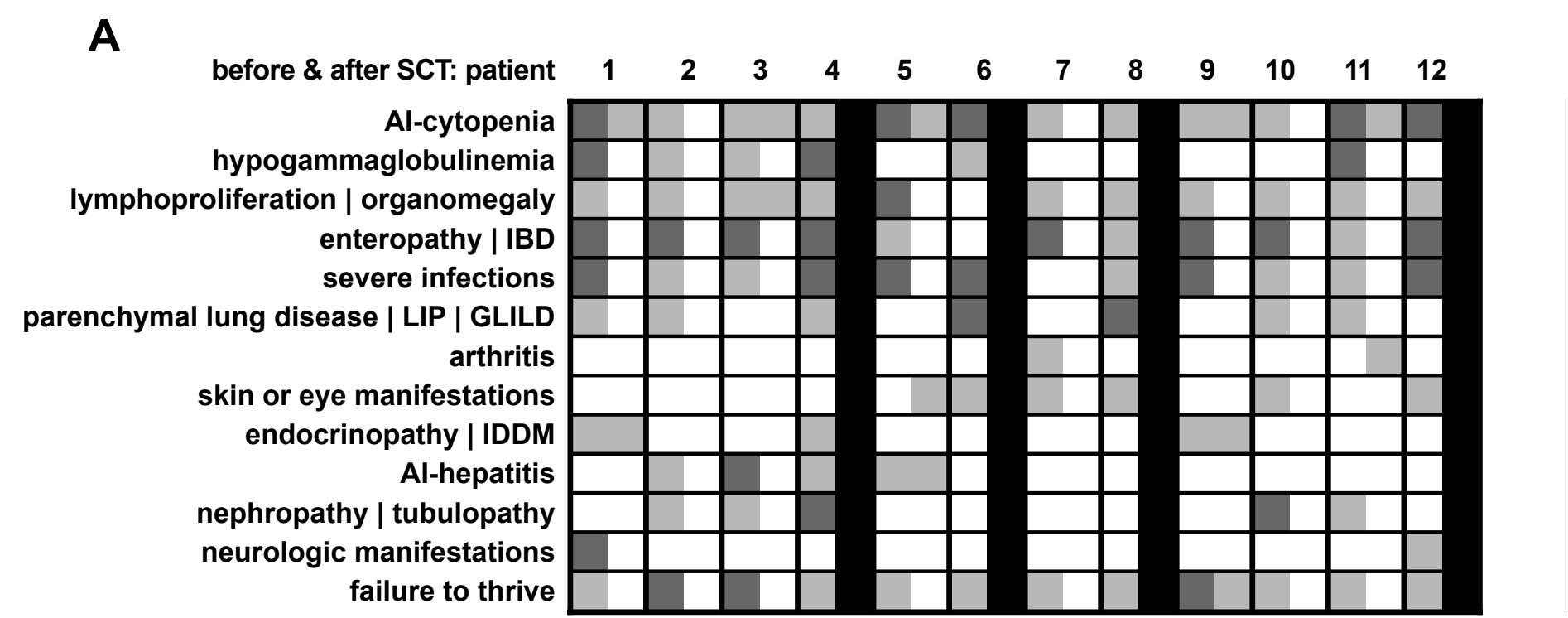
Patient characteristics	1	2	3	4	5	6	7	8	9	10	11	12
mutation	c.7162delA	c.5505delT	c.675G>A	c.1420G>A; c.2834_2837 delTCTT	c.7162delA	c.2004+2A>G	c.3647_3651 delCTAA; c.7937T>G	Exon 1-30 deletion	c.2978C>G	c.4522 C>T	c.7937T>G	c.2762G>C
age at onset (yrs)	7	1	3	3	2	3	0,1	4	1	3	1	4
Previous publication	[7]	[8]	*	[1]	[1; 6]	[1]	[9]	[1]	*	*	*	*
LRBA-directed treatment before SCT												
steroids	X	X	X	X	X	X	X	X	X	X	X	X
rituximab					X		X	X	X	X		X
abatacept											X	
sirolimus				X	X		X			X	X	
hydroxychloroquine										X	X	
MMF			X					X	X	X		
azathioprine	X					X	X			X		
other IS			CSA/FK	CSA/FK, Infliximab		CSA/FK, Basilixi-mab	MTX, CSA/FK			CSA/FK		not specified
IVIG	X	X	X	X	X	X	X		X	X	X	X
SCIG	X		X					X	X			
SCT conditioning & course												
Indication for SCT	severe CVID-related clinical problems, ADEM	severe clinical course un-responsive to treatment	severe clinical course un-responsive to treatment	chronic interstitial lung disease, chronic inflamma-tory bowel disease	refractory pancyto-penia and lympho-proliferation	Recurrent respiratory infections & insufficiency, pancyto-penia, lympho-proliferation	enteropathy, auto-immunity	Steroid refractory lymphocytic insterstitial pneumonia	severe clinical course, enteropathy, infections, failure to thrive	Refractory CNS lympho-proliferation	Lympho-proliferation	severe humoral immuno-deficiency, intestinal problems, auto-immunity
age at HSCT (yrs)	13	12	7	11	10	6	11	10	3	16	9	10
year of SCT	2013	2014	2015	2015	2005	2013	2010	2012	2015	2015	2016	2009
donor type (& LRBA status)	MFD (het)	MSD (n.d.)	MSD (het)	MUD (n.d.)	MFD (het)	MMFD (het)	MSD (het)	MUD (n.d.)	MFD (wt)	MUD (n.d.)	MSD (het)	MUD (n.d.)

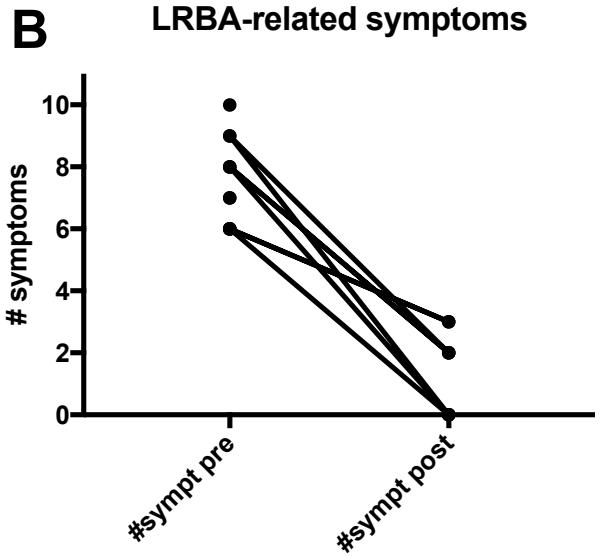
Table 1. Clinical characteristics and laboratory parameters of 12 LRBA-deficient patients who underwent allogeneic hematopoietic stem cell transplantation (HSCT).

Patients	1	2	3	4	5	6	7	8	9	10	11	12
SCT conditioning & course (continued)												
Fludarabin & serotherapy	X	X	X	X	X	X	X	X	X	X	X	X
Treosulfan	X			X					X		X	
Busulfan		X	X							X		
Thiotepa				X		X	X					
Melphalan					X	X	X	X				X
aGvHD						1° (skin)	1°-2° (skin)					
cGvHD										limited: eye, gut		
peri-SCT infections				aspergillosis & CMV (pre-SCT)		AdV		AdV, CMV				
post-SCT complications / TRM and cause of death				invasive aspergillosis	post-SCT autoimmune hepatitis, successfully treated with AZT and resolved	respiratory failure, lung fibrosis, granuloma, adenovirus pneumonia, graft rejection		TMA, AdV viremia, pneumonitis	Post-SCT AIHA		still requires hemato-poietic growth factor support, may need SC boost	poor engraftment, CMV pneumonitis, Enterobacter and Acineto-bacter sepsis
outcome												
overall survival	X	X	X		X		X		X	X	X	
FU (months)	36	25	27	*(3)*	142	*(3)*	75	*(2)*	22	9	4	*(2)*
Donor chimerism (% WBC unless otherwise stated)	>95 T, B, myeloid (+3yrs)	98 (+2yrs)	83 WBC, 84 CD3+ (+2yrs$^{\$}$)	100 (d+100)	100 (+3yrs)	n.a.	100 (+3yrs)	100 (d+30)	7 WBC, 23 CD3+ (d+578$^{\$}$)	97 WBC, 44 CD3+ (d+180)	100 (d+120)	0 (d+30)
remission status	GPR (mild thrombocyto penia and thyroid disease)	CR	PR (moderate cITP and AIHA)	died 3m post-SCT (TRM)	GPR (mild cITP, vitiligo)	died 3m post-SCT (TRM)	CR	died 2m post-SCT (TRM)	PR (AIHA; irreversible IDDM after pancreatitis)	CR	GPR (thrombocyt openia, neutropenia, flares of arthritis)	died 2m post-SCT (TRM)
current treatment	none	none	TRA, sirolimus, IVIG	n.a.	TRA (recently discont'd)	n.a.	none	n.a.	abatacept IVIG	none	TRA, GCSF	n.a.

Table 1. Clinical characteristics and laboratory parameters of 12 LRBA-deficient patients who underwent allogeneic hematopoietic stem cell transplantation (HSCT).

* manuscripts (single patient reports) in preparation (*personal communication*).
[$] declining donor chimerism in patient 3 from 95-99% donor WBC with 91% donor T cells (days +30, +60) to 89% WBC and 88% CD3+ (day+240), and further decreasing to 83% WBC and 84% T cell chimerism two years after HSCT; and in patient 9 from 100% donor WBC days +30 and +60 to 93% on day +82, which further declined to 15% WBC and 31% CD3+ on day+430 and down to 7% donor-derived WBC and 23% CD3+ T cells day +578 at the latest follow-up (as shown).


A
before & after SCT: patient
1
2
3
4
5
6
7
8
9
10
11
12
AI-cytopenia
hypogammaglobulinemia
lymphoproliferation | organomegaly
enteropathy | IBD
severe infections
parenchymal lung disease | LIP | GLILD
arthritis
skin or eye manifestations
endocrinopathy | IDDM
AI-hepatitis
nephropathy | tubulopathy
neurologic manifestations
failure to thrive


B
LRBA-related symptoms
symptoms
10
8
6
4
2
0
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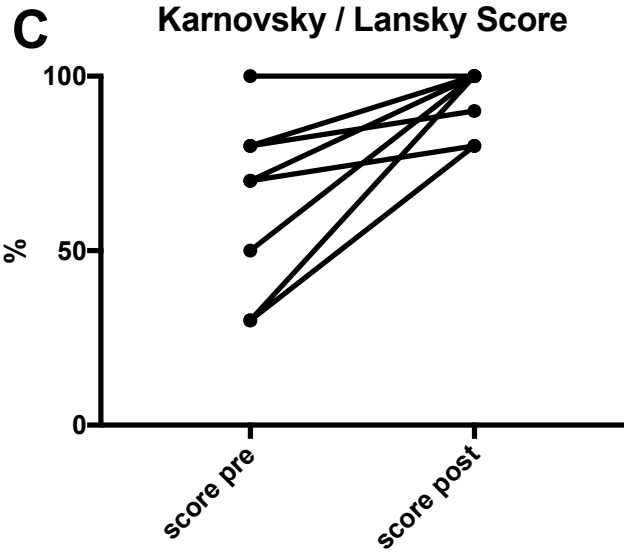
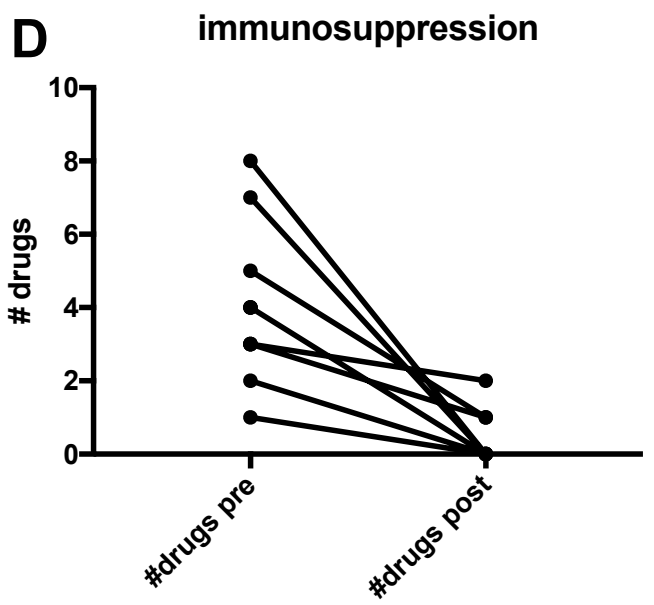
#sympt post


C
Karnovsky / Lansky Score
%
100
50
0
score pre
score post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drugs
10
8
6
4
2
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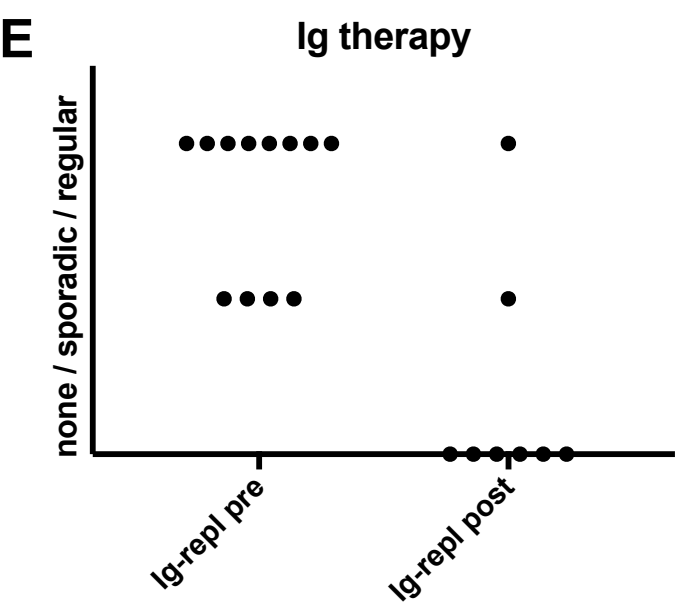
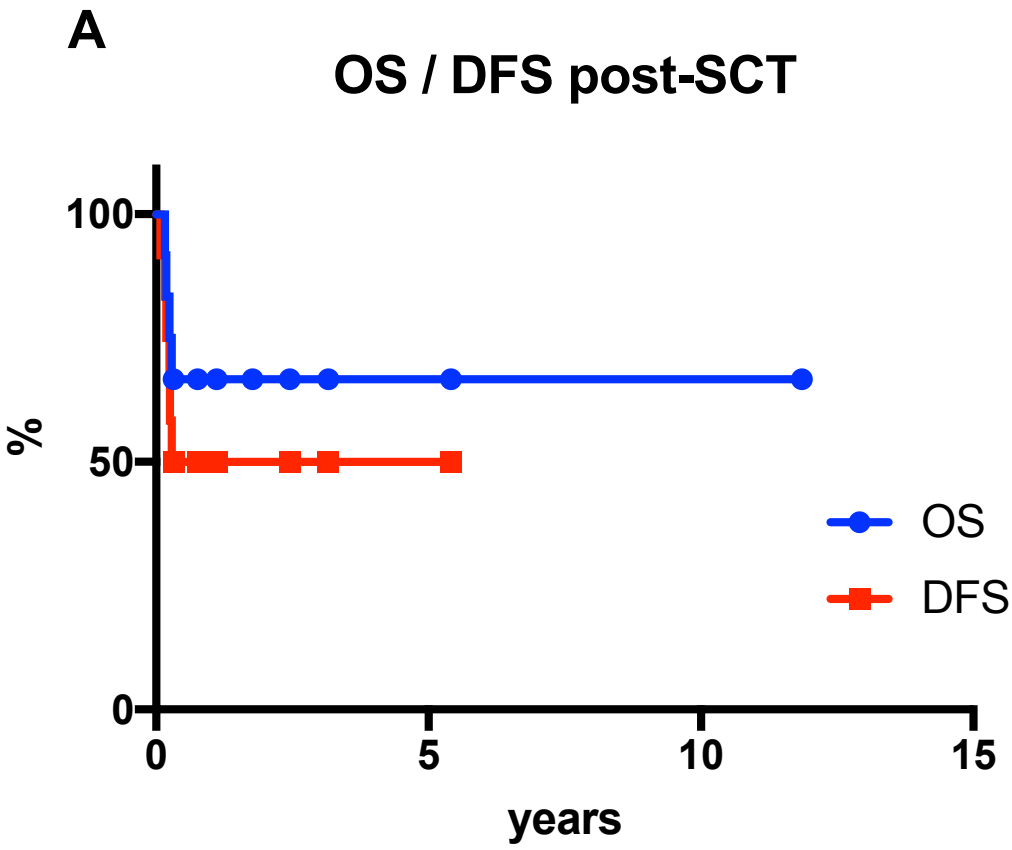
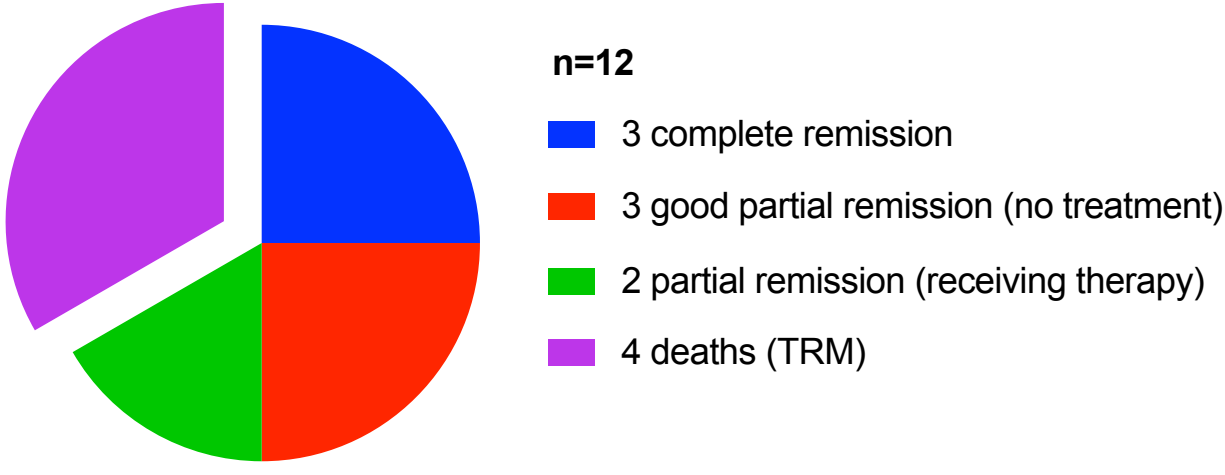
#drugs post


E
Ig therapy
none / sporadic / regular
Ig-repl pre
Ig-repl post

A


OS / DFS post-SCT
100
50
0
%
0
5
10
15
years
OS
DFS

B


n=12
3 complete remission
3 good partial remission (no treatment)
2 partial remission (receiving therapy)
4 deaths (TRM)